

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 January 2003 (30.01.2003)

PCT

(10) International Publication Number
WO 03/008971 A2

(51) International Patent Classification⁷: **G01N 33/53**

(74) Agents: FISHMAN, Bella et al.; Varian, Inc., Legal Department, D-102, 3120 Hansen Way, Palo Alto, CA 94304 (US).

(21) International Application Number: **PCT/US02/22668**

(81) Designated States (*national*): AU, JP.

(22) International Filing Date: 16 July 2002 (16.07.2002)

(84) Designated States (*regional*): European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR).

(25) Filing Language: English

Published:

(26) Publication Language: English

— without international search report and to be republished upon receipt of that report

(30) Priority Data:
09/910,198 20 July 2001 (20.07.2001) US

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

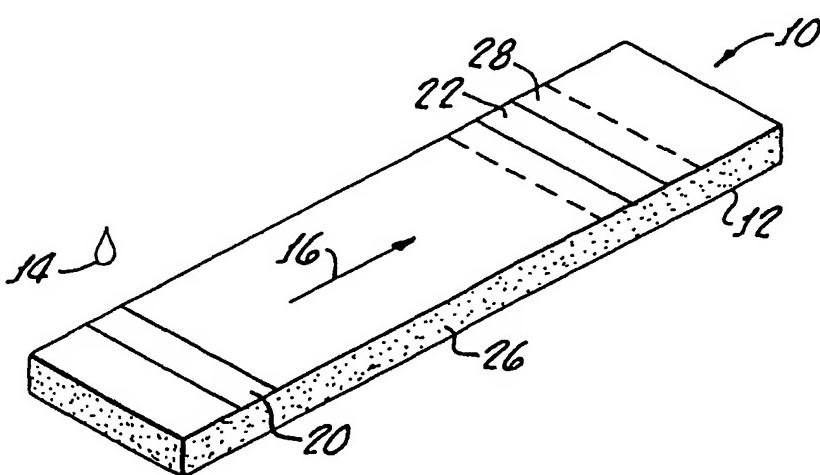
(71) Applicant: VARIAN, INC. [US/US]; 3120 Hansen Way, D-102, Palo Alto, CA 94304 (US).

(72) Inventors: SIDWELL, Steven, P.; 33482 Dosinia Drive, Dana Point, CA 92629 (US). BACHAND, Steven, S.; 23767 North Shore Lane, Laguna Niguel, CA 92677 (US).



WO 03/008971 A2

(54) Title: COLOR CONTRAST SYSTEM FOR LATERAL FLOW IMMUNOASSAY TESTS



(57) Abstract: A lateral flow immunoassay device includes a membrane strip for enabling capillary migration of a sample therealong with a labeled reagent disposed on the membrane. The label reagent is formulated for suspension in the sample migrating therewith. A captive reagent is immobilized on the strip on a path of sample migration and the captive reagent is formulated to bind to the labeled reagent to form a visible colored site on the strip. An element is provided for changing the strip to a color which enhances visual perception of the colored site.

BEST AVAILABLE COPY

COLOR CONTRAST SYSTEM FOR LATERAL FLOW
IMMUNOASSAY TESTS

FIELD OF THE INVENTION

The present invention generally relates to solid phase immunoassay test devices whether the sandwich or competition type for providing sensitive detection of an analyte in a biological fluid sample and is more particularly directed to a colored contrast system therefore. Solid phase immunoassay test devices incorporate a solid support to which one member of the ligand-receptor pair, usually an antibody, antigen, or hapten is bond.

BACKGROUND OF THE INVENTION

Lateral flow immunoassay tests in typical use today, generally include a porous component of nitrocellulose membrane, as the solid, with specific reagent applied onto specific zones therein.

An upstream zone usually includes a specific binding reagent for the analyte being tested, conjugated to a visible label such as a gold colloid or colored latex particle. The labeled reagent is formulated to facilitate its released from the membrane after the sample is applied to the test strip.

In typical competition assays, a sample containing analyte is introduced to a sample area of the test strip. Migration of the sample, caused by the capillary wicking within the porous membrane, re-suspends the labeled reagent from its stationary position on the strip. As the reagent mixture migrates along the strip it is brought into contact with the immobilized capture reagent. If the analyte is present in the sample, binding to the labeled reagent (Antibody-visual label) will take place during this migration.

If the amount of analyte is enough to exhaust all binding sites on the labeled reagent, binding of the visual label to the capture reagent will not occur. This constitutes a positive result.

If there is no analyte in the sample, the visual label will bind at the capture stripe producing a negative result seen as a colored band or strips.

For the most part, these tests are interpreted visually by human eye to determine the presence or absence of an analyte (drug). Membranes like nitrocellulose provide a white background to visualize the presence or absence of the colored line or stripe. Presently, white is the only color commercially available for nitrocellulose membrane.

Partial sight, aging, and congenital color deficits can produce changes in perception that reduce the visual effectiveness or certain color combinations.

The present invention provides for lateral flow immunoassay device utilizing complementary colors to provide better contrast for visual perception of test results.

SUMMARY OF THE INVENTION

A lateral flow immunoassay device in accordance with the present invention generally includes a porous strip for enabling capillary migration of a fluid sample therealong. A labeled reagent is disposed on the stripe with the label reagent being formulated for suspension in the sample migrating therewith. A captive reagent is immobilized on the strip in a path of the sample migration with the captive reagent being formulated to bind to the labeled reagent to form a visual colored site on the strip.

Also included are means for providing a complimentary color background for the colored site in order to increase visual perception of the colored site.

In one embodiment of the present invention, the means for providing a complimentary color background comprises a dye incorporated into the porous strip. In another embodiment of the present invention a means for providing a complementary color background comprises a transparent colored film disposed over the porous strip. The film may be in direct contact with the strip by adhesive or suspended over the strip in close proximity therewith with minimal or no contact with the strip.

When a white porous nitrocellulose membrane is used for the porous strip, the means in accordance with the present invention is, in effect an element for changing the white strip to a color which enhances a visual perception of the colored site.

The nitrocellulose membrane may be formed on a Mylar backing, as is well known in the art. In the case of the present invention this Mylar backing may be an optically clear yellow Mylar and membrane viewed through the Mylar backing as will be discussed hereinafter.

In the embodiment in which a film is utilized, the latter may be laminated or adhered to the porous strip or spaced apart therefrom as hereinabove noted.

More specifically, when the colored site is blue, the complementary color background may be selected from a group consisting of yellow, yellow-orange and orange.

Alternative complimentary color combination that may be utilized such as a green background when the colored site is red.

The present also provides for an improvement in existing lateral flow immunoassay devices having a strip for enabling capillary migration of a fluid sample therethrough, a label reagent disposed on the strip and formulated for suspension in the sample migrating therewith, and a captive agent immobilized on the strip in a path of sample migration and formulated to bind with the label reagent to form a visible site on the strip. The improvement comprises a colored background for enhancing the color perception of the colored site which, may be, for example, a dye incorporated into the strip or a transparent film of a selected background color either directly laminated to the nitrocellulose strip by pressure sensitive adhesive or suspended barely above the strip with minimal or no contact with the strip, facilitated by a die-cut thin plastic carrier, or the like.

A method in accordance with the present invention for enhancing visual perception of a colored site in a immunoassay device includes dying the strip a color which is complimentary to the colored site produced by binding of the labeled reagent and the capture reagent or the step of providing a transparent film having a color which is complimentary to the colored site.

BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and features of the present invention will be better understood by the following description when considered in conjunction with the accompanying drawings in which:

Figure 1 is a perspective view of a lateral immunoassay in accordance with the present invention generally showing a porous strip, a label reagent disposed on the strip along with a captive reagent immobilized on the strip and a representation of dye present in the strip for providing a color background as will be hereinafter described in greater detail; and

Figure 2 is a cross-sectional view of another embodiment of the present invention showing a porous strip and representations of a labeled reagent disposed on the strip, a captive reagent immobilized on the strip and a transparent film for providing a complimentary background; and

Figure 3 is an alternative embodiment of the present invention utilizing a nitrocellulose membrane on a colored backing.

DETAILED DESCRIPTION OF THE INVENTION

With reference to Figure 1, there is shown a lateral flow immunoassay device 10 in accordance with the present invention which generally includes a porous strip 12, which typically is a nitrocellulose membrane or the like, which enables capillary migration of a fluid sample 14 therewith as represented by the arrow 16 after the deposition of the sample 14 onto the strip 12. The deposition preferably occurs on an area 20 of the strip where a labeled reagent is disposed. The labeled reagent is formulated, as is well known in the art, for suspension in the sample migrating therewith. A captive reagent is immobilized on the strip at a second zone 22 and formulated to bind to the labeled reagent to form a visible site on the strip.

In the embodiment 10 shown in Figure 1, a dye 26, which is preferably indelible, is incorporated as an element which acts as a means for providing a complimentary color background for the colored site in order to increase visual perception of the colored site. Complimentary colors are those which appear generally opposite one another on a conventional color wheel which include the primary colors of yellow, blue and red.

Typical labeled reagents, such as, blue latex microparticles conjugated to drug antibody, and captive reagents such as, immobilized drug conjugates, result in a blue site. Complementary colors for blue are yellow and orange which are "warm" colors that can optically move the subject, i.e., the blue colored site to the foreground.

As hereinabove noted color compliments are color opposites. They are opposite each other on the color wheel, for example, blue is opposite orange and yellow. These colors are in extreme contrast to each other while making each more intense, for example, a bright orange or yellow background will highlight and make blue more vibrant.

This is an advantage to the visual interpretation of a lateral flow test when the signal to be interpreted becomes faint to the eye due to the quantity of analyte. In competitive assays, low amounts of analyte, under the proposed cut-off of the test, will weaken the visual signal to the point of producing a false positive. The present invention provides for a color contrast system that makes the color signal easier to see. In typical "sandwich" assays in which a colored line indicates a positive sample, the color contrast system in accordance with the present invention helps prevent false negatives particularly in persons with color vision defects.

While a permanent dye 26 may be utilized in accordance with the present invention, it should be appreciated that chemical components may be added to the strip which in fact cause a color background to be developed at the same time as binding of the labeling reagent

in the captive reagent to form the colored site. For example, an anti BSA captive zone 28 may be provided under the capture zone 22, and broader thereof, and yellow latex microparticles introduced that have immobilized BSA on the surface with the blue latex microparticulates. As the sample runs, the yellow microparticulates will stop at the BSA captive zone 28, making it yellow, and if a drug was not present the blue microparticulates will stop at the captive zone 22.

Alternatively, as shown in Figure 2 a second embodiment 30 of a lateral flow immunoassay device in accordance with the present invention, includes a porous strip 32 for enabling a sample to migrate therewith by capillary action as indicated by the arrow 36.

A labeled reagent 40 is disposed on the membrane and formulated as hereinabove noted for suspension in the sample 34 for migrating therewith. A captive reagent 42 is immobilized in the strip 32 in the path of sample migration and formulated to bind with the label reagent to form a visible colored site on the strip.

In the embodiment 30 the strip 32 may be a white porous membrane and a transparent film 50 of a selected color is disposed over the membrane strip 32. In order to provide a unitary device 30, the film 50 may be laminated to the membrane strip 32.

Alternatively, the film 50, which may be in the form of a plastic carrier or encasement 52, may be suspended above the strip 32 and include a transparent pigment for allowing light and the underlining test strip 32 to be visualized.

An alternative embodiment 60 of the present invention is shown in Figure 3. Common references characters shown in Figure 3 correspond to identical or similar reference characters shown in Figures 1-2.

The embodiment 60 includes the porous membrane 32 disposed on a transparent colored backing 62 preferably yellow Mylar. Alternatively a clear Mylar backing may be used with a transparent colored film 64, thereon as indicated in dashed line in Figure 3. In this embodiment, the membrane 32 is viewed through the Mylar 62 as indicated by the icon 66.

EXPERIMENTAL RESULTS

Lateral flow test strips were challenged with various levels of analytes and specimens. The levels were focused around the detection limits of the test device. The blue latex colored particles were used for the test. Two sets of identical test strips were challenged with the specimens. One set of the test strips were covered with a yellow Mylar strip. Multiple individuals made visual reads of the strips and the readers commented on the ease of reading

strips when the yellow film covered the test strip which enhanced their ability to interpret the presence or absence of the blue line result.

Although there has been hereinabove described a lateral flow immunoassay device in accordance with the present invention utilizing a specific complimentary colors of yellow and blue, it should be appreciated that the invention is not limited thereto but further incorporates the use of any sets of complimentary colors for enhancing visual equity of test results. Accordingly, any and all modifications, variations, or equivalent arrangements which may occur to those skilled in the art should be considered to be within the scope of the invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. A lateral flow immunoassay device comprising:
a porous strip for enabling capillary migration of a fluid sample therealong;
a labeled reagent disposed on the strip, said labeled reagent being formulated for suspension in the sample migrating therewith;
a captive reagent immobilized on the strip in a path of sample migration, said captive reagent being formulated to bind to said labeled reagent to form a visible colored site on the strip; and
means for providing a complimentary color background for the colored site in order to increase visual perception of the colored site.
2. The device according to claim 1 wherein the means for providing a complimentary color background comprises dye incorporated into said porous strip.
3. The device according to claim 1 wherein the means for providing a complimentary color background comprises a transparent colored film disposed over said porous strip.
4. The device according to claim 3 wherein the film is suspended above said porous strip.
5. The device according to claim 3 wherein the film is laminated to said porous strip.
6. The device according to any one of claim 1 through 5 wherein the colored site is blue and the complimentary color background is selected from a group consisting of yellow, yellow-orange and orange.
7. The device according to any one of claims 1 through 4 wherein the colored site is red and the complimentary color background is selected from a group consisting of green, light green, fluorescent green and lime green.
8. A lateral flow immunoassay device comprising:
a white porous nitrocellulose membrane for enabling capillary migration of a fluid sample therealong;

a labeled reagent disposed on the membrane, said labeled reagent being formulated for suspension in the sample migrating therewith;

a captive reagent immobilized on the strip in a path of sample migration, said captive reagent being formulated to bind to said labeled reagent to form a visible colored site on the strip; and

an element for changing the white strip to a color which enhances visual perception of said colored site.

9. The device according to claim 8 wherein the element comprises a dye incorporated into the membrane.

10. The device according to claim 8 wherein the element comprises a transparent film disposed over the membrane.

11. The device according to claim 10 wherein said transparent film is laminated to the membrane.

12. The device according to claim 8 wherein said element comprises a colored backing for supporting the membrane.

13. The device according to claim 8 wherein said element comprises a clear backing for supporting the membrane and a transparent colored film adhered to said clear backing.

14. The device according to any one of claims 8 through 13 wherein the colored site is blue and the enhancing color is selected from a group consisting of yellow, yellow-orange and orange.

15. The device according to any one of claims 8 through 13 wherein the colored site is red and the enhancing color is selected from a group consisting of green, light green, fluorescent green and lime green.

16. An improvement in a lateral flow immunoassay device having a strip for enabling capillary migration of a fluid sample therewith, a labeled reagent disposed on the strip and formulated for suspension in the sample migrating therewith and a captive reagent

immobilized on the strip in a path of sample migration and formulated to bind to said labeled reagent to form a visible colored site on said strip, said improving comprising a color background for enhancing visual perception of said colored site.

17. The improvement according to claim 16 wherein said color background comprises a dye fixed in said strip.

18. The improvement according to claim 16 wherein said color background comprises a transparent film disposed over said strip.

19. The improvement according to any one of claims 13 through 18 wherein said colored site is blue and said colored background is yellow.

20. The improvement according to any one of claims 13 through 18 wherein said colored site is red and said colored background is green.

21. A method for enhancing visual perception of colored site in an immunoassay device, the device comprising a strip for enabling capillary migration of a fluid sample therealong, a labeled reagent disposed on the strip and formulated for suspension in the sample migrating therewith and a capture reagent immobilized on said strip in a path of sample migration and formulated to bind to said labeled reagent to form said colored site, said method comprising dyeing said strip a color which is complimentary to said colored site.

22. A method for enhancing visual perception of a colored site in an immunoassay device, the device comprising a strip for enabling capillary migration of a fluid sample therealong, a labeled reagent disposed on the strip and formulated for suspension in the sample migration therewith and a capture reagent immobilized on said strip in a path of sample migration and formulated to bind to said labeled reagent to form said colored site, said method comprising coloring said strip with a transparent film having a color which is complementary to said colored site.

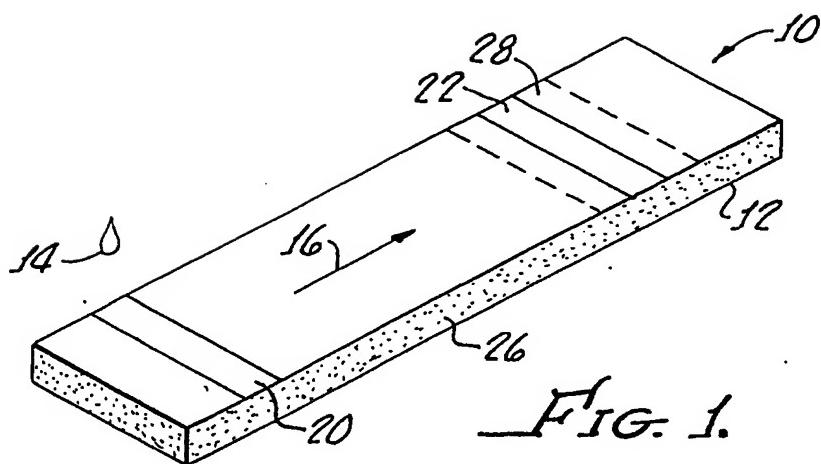


FIG. 1.

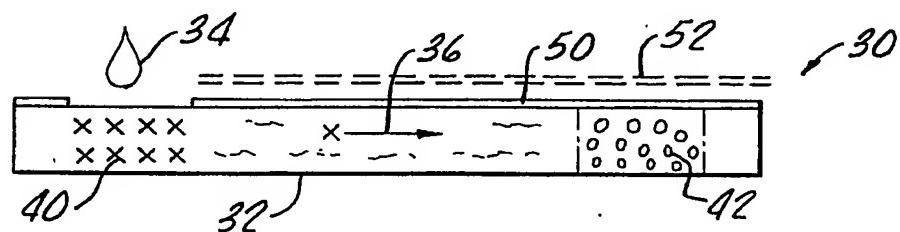


FIG. 2.

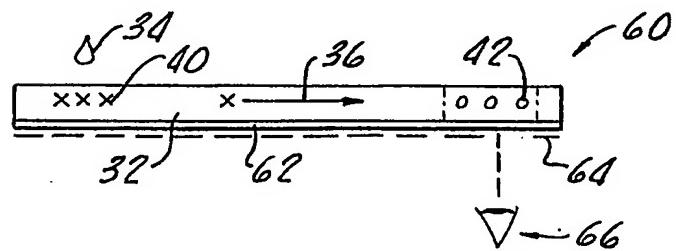


FIG. 3.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 January 2003 (30.01.2003)

PCT

(10) International Publication Number
WO 03/008971 A3

(51) International Patent Classification⁷: **G01N 33/558** (74) Agents: FISHMAN, Bella et al.; Varian, Inc., Legal Department, D-102, 3120 Hansen Way, Palo Alto, CA 94304 (US).

(21) International Application Number: PCT/US02/22668

(22) International Filing Date: 16 July 2002 (16.07.2002)

(81) Designated States (*national*): AU, JP.

(25) Filing Language:

English

(84) Designated States (*regional*): European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR).

(30) Priority Data:

09/910,198 20 July 2001 (20.07.2001) US

Published:

— with international search report

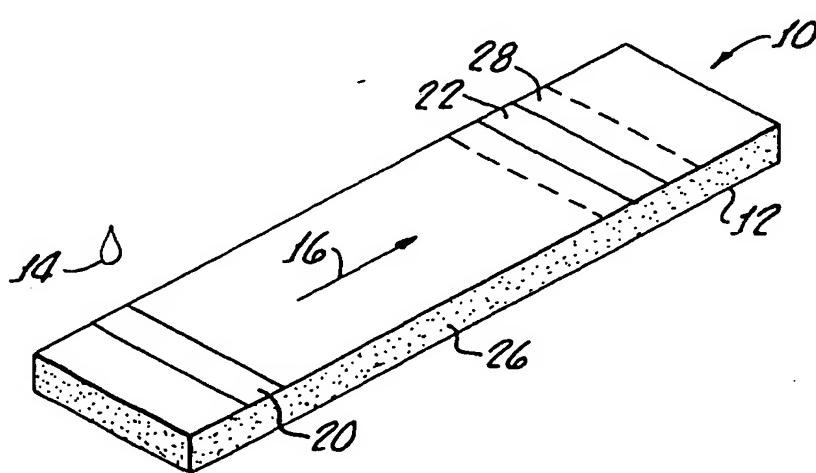
(71) Applicant: VARIAN, INC. [US/US]; 3120 Hansen Way, D-102, Palo Alto, CA 94304 (US).

(88) Date of publication of the international search report:
9 October 2003

(72) Inventors: SIDWELL, Steven, P.; 33482 Dosinia Drive, Dana Point, CA 92629 (US). BACHAND, Steven, S.; 23767 North Shore Lane, Laguna Niguel, CA 92677 (US).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COLOR CONTRAST SYSTEM FOR LATERAL FLOW IMMUNOASSAY TESTS



(57) Abstract: A lateral flow immunoassay device includes a membrane strip for enabling capillary migration of a sample therealong with a labeled reagent disposed on the membrane. The label reagent is formulated for suspension in the sample migrating therewith. A captive reagent is immobilized on the strip on a path of sample migration and the captive reagent is formulated to bind to the labeled reagent to form a visible colored site on the strip. An element is provided for changing the strip to a color which enhances visual perception of the colored site.

WO 03/008971 A3

INTERNATIONAL SEARCH REPORT

International application No

PCT/US 02/22668

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/558

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 27627 A (IMP COLLEGE INNOVATIONS LTD ;CRISANTI ANDREA (GB); FRIEDLANDER URI) 19 April 2001 (2001-04-19) page 41, paragraph 1 ---	1-22
X	US 5 877 028 A (CHANDLER HOWARD M ET AL) 2 March 1999 (1999-03-02) column 34, line 40 - line 46 ---	1-22

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- °A° document defining the general state of the art which is not considered to be of particular relevance
- °E° earlier document but published on or after the International filing date
- °L° document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- °O° document referring to an oral disclosure, use, exhibition or other means
- °P° document published prior to the international filing date but later than the priority date claimed

- °T° later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- °X° document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- °Y° document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- °&° document member of the same patent family

Date of the actual completion of the international search

28 May 2003

Date of mailing of the International search report

10/06/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Stricker, J-E

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US 02/22668

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 0127627	A	19-04-2001	AU CA EP WO	7805900 A 2395057 A1 1222462 A2 0127627 A2	23-04-2001 19-04-2001 17-07-2002 19-04-2001
US 5877028	A	02-03-1999	US AT AU AU BG BR CA CN DE DE DK EP ES FI HU JP NO NZ OA PL RU SK WO US US US US US US US US US US US AT AU AU CA DE DE DK EP EP ES FI JP JP WO	6168956 B1 177206 T 678461 B2 6497094 A 100104 A 9406755 A 2158570 A1 1124524 A 69416828 D1 69416828 T2 692097 T3 0692097 A1 2131191 T3 954591 A 73379 A2 8508569 T 953872 A 263754 A 10233 A 310953 A1 2124729 C1 122795 A3 9423300 A1 5468648 A 5607863 A 5869345 A 5648274 A 5846838 A 5998220 A 6017767 A 174432 T 665956 B2 2185292 A 2103052 A1 69227834 D1 69227834 T2 586595 T3 0586595 A1 0874241 A1 2127754 T3 935244 A 3386122 B2 6508215 T 9221977 A1	02-01-2001 15-03-1999 29-05-1997 24-10-1994 31-05-1996 02-04-1996 13-10-1994 12-06-1996 08-04-1999 08-07-1999 04-10-1999 17-01-1996 16-07-1999 27-11-1995 29-07-1996 10-09-1996 06-11-1995 24-03-1997 07-10-1997 08-01-1996 10-01-1999 05-06-1996 13-10-1994 21-11-1995 04-03-1997 09-02-1999 15-07-1997 08-12-1998 07-12-1999 25-01-2000 15-12-1998 25-01-1996 08-01-1993 30-11-1992 21-01-1999 29-04-1999 16-08-1999 16-03-1994 28-10-1998 01-05-1999 25-11-1993 17-03-2003 14-09-1994 10-12-1992

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.